Angewandte Chemie

Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

A Chemical Perspective on Transcriptional Fidelity: Dominant Contributions of Sugar Integrity Revealed by Unlocked Nucleic Acids**

*Liang Xu, Steven W. Plouffe, Jenny Chong, Jesper Wengel, and Dong Wang**

anie_201307661_sm_miscellaneous_information.pdf

Supporting Information

Experimental Details

Materials. *Saccharomyces cerevisiae* RNA Pol II was purified as previously described.[1] The DNA template and non-template oligonucleotides were purchased from IDT. RNA primers were purchased from TriLink Biotechnologies. All nucleic acid oligos were PAGE and HPLC purified. RNA primers were radiolabelled using γ-^{32}P-ATP and T4 Polynucleotide Kinase (NEB).

In vitro **transcription assays.** The Pol II elongation complexes for transcription assays were assembled using established methods.[2] Briefly, an aliquot of 5'-^{32}P-labeled RNA was annealed with a 1.5-fold amount of template DNA and 2-fold amount of non-template DNA to form the RNA/DNA scaffold in elongation buffer (20 mM Tris-HCl, pH 7.5, 40 mM KCl, 5 mM $MgCl_2$). An aliquot of the annealed scaffold of RNA/DNA was then incubated with a 4-fold excess amount of Pol II at room temperature for 10 min to ensure the formation of a Pol II elongation complex. The Pol II elongation complex is ready for *in vitro* transcription upon mixing with equal volumes of nucleotide solution containing various concentrations of ATP, ***a***TP, UTP, ***u***TP, CTP, or NTP solution. The quenched products were analyzed by denaturing PAGE and visualized using a storage phosphor screen and Pharos FX imager (Bio-Rad). Substrate and product bands were quantified using Image Lab 3.0. Scaffolds for transcription assays were described in Figure S1.

Single-turnover nucleotide incorporation assays. The assay was carried out as previously described.[2] Briefly, nucleotide incorporation assays were conducted by pre-incubating 50 nM scaffold with 200 nM Pol II for 10 min in elongation buffer at 22 °C. The pre-incubated enzyme:scaffold complex was then mixed with an equal volume of solution containing 40 mM KCl, Tris (pH = 7.5), 10 mM DTT, 10 mM $MgCl_2$, and 2-fold concentrations of various nucleotides. Final reaction concentrations after mixing were 25 nM scaffold, 100 nM Pol II, 5 mM $MgCl_2$, and various nucleotide concentrations in elongation buffer. Reactions were quenched at various times by addition of one volume of 0.5 M EDTA (pH = 8.0).

TFIIS cleavage assays. Recombinant transcription factor IIS (TFIIS) was purified as described.[2] Cleavage reactions were performed by pre-incubating Pol II with various scaffolds as previously described with slight modification. The solution was then mixed with an equal volume of solution containing TFIIS and $MgCl_2$ in elongation. Final reaction conditions were 100 nM Pol II, 25 nM scaffold, 1.5 μM TFIIS, and 5 mM $MgCl_2$. Reactions were quenched at various time points and products were analyzed as described.[2]

Data analysis. Nonlinear-regression data fitting was performed using Prism 6. The time dependence of product formation was fit to a one-phase association equation (1) to determine the observed rate (k_{obs}). The substrate concentration dependence was fit to a hyperbolic equation (2) to obtain values for the maximum rate of NTP

incorporation (k_{pol}) and apparent K_d ($K_{d,app}$) governing NTP binding (See representative kinetic fittings in Figure S5).

$$\text{Product} = Ae^{(-k_{obs}\, t)} + C \quad (1)$$

$$k_{obs} = k_{pol}\,[\text{Substrate}] / (K_{d,app} + [\text{Substrate}]) \quad (2)$$

The specificity constant was determined by $k_{pol}/K_{d,app}$. which is derived from the rate of polymerization at the active site (k_{pol}) and apparent dissociation constant for nucleotide binding ($K_{d,app}$). The specificity constant is the measurement of the substrate incorporation efficiency of an enzyme. Discrimination was calculated from the ratio of the $k_{pol}/K_{d,app}$ values of substrate A over substrate B ($(k_{pol}/K_{d,app})_A/ (k_{pol}/K_{d,app})_B$) as described.[2] Discrimination is a quantitative measure of the enzyme's discrimination power of two related substrates.

Supplementary Table

Table S1. Effect of Sugar Backbone Integrity on RNA Pol II Transcription and Elongation.

Primer	NTP	k_{pol} (min^{-1})	$K_{d,app}$ (μM)	$k_{pol}/K_{d,app}$ ($\mu M^{-1}\cdot min^{-1}$)	Substrate Discrimination[a]	Primer Discrimination[b]
10A	ATP	750 ± 210	90 ± 20	8.3 ± 3.0	-	-
	*a*TP	$(5.8 \pm 0.2) \times 10^{-4}$	940 ± 100	$(6.2 \pm 0.7) \times 10^{-7}$	$(1.3 \pm 0.5) \times 10^{7}$	-
10*a*$_n$	ATP	$(4.3 \pm 0.1) \times 10^{-3}$	93 ± 12	$(4.6 \pm 0.6) \times 10^{-5}$	-	$(1.8 \pm 0.7) \times 10^{5}$
10*g*$_{n-1}$	ATP	5.7 ± 0.2	58 ± 6.4	$(9.8 \pm 1.1) \times 10^{-2}$	-	85 ± 31
10*g*$_{n-2}$	ATP	5.7 ± 0.1	3.6 ± 0.6	1.6 ± 0.3	-	5.2 ± 2.1

[a] Discrimination = $(k_{pol}/K_{d,app})_{ATP}$ / $(k_{pol}/K_{d,app})_{aTP}$; [b] Discrimination = $(k_{pol}/K_{d,app})_{10A}$ / $(k_{pol}/K_{d,app})_{10an \text{ or } 10gn-1 \text{ or } 10gn-2}$

Supplementary Figures

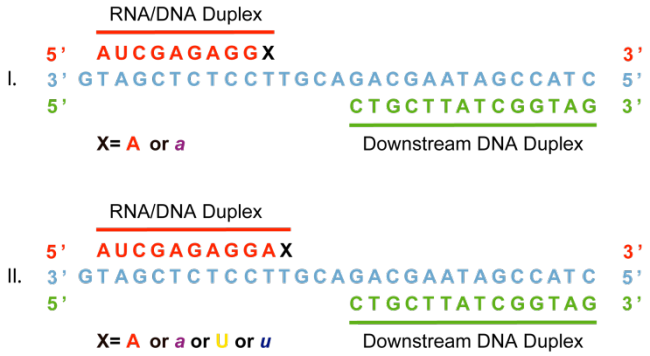

Figure S1. Scaffold sequences of RNA, template DNA, and non-template DNA in this study. Scaffold I (10A or 10$\boldsymbol{a}_n$) contains a 10mer RNA primer, and Scaffold II (11A, 11$\boldsymbol{a}$, 11U, or 11$\boldsymbol{u}$) contains an 11mer RNA primer. X refers to nucleotides or unlocked nucleotides. The unlocked nucleotides are denoted in bold italic lowercase.

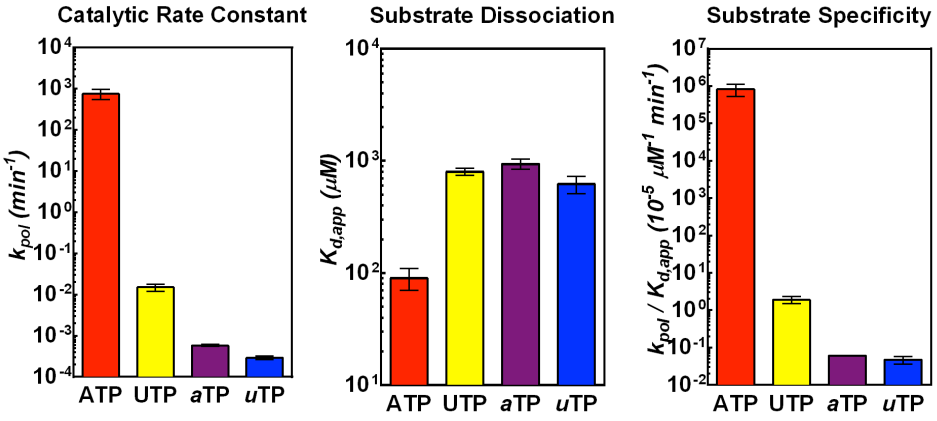

Figure S2. Nucleotide incorporation specificity for canonical and unlocked nucleotides (the first checkpoint step). Rate constants (k_{pol}), substrate dissociation constants ($K_{d,app}$) and substrate specificity constants ($k_{pol}/K_{d,app}$) for ATP, UTP, ***a***TP and ***u***TP incorporation are shown in red, yellow, violet and blue, respectively.

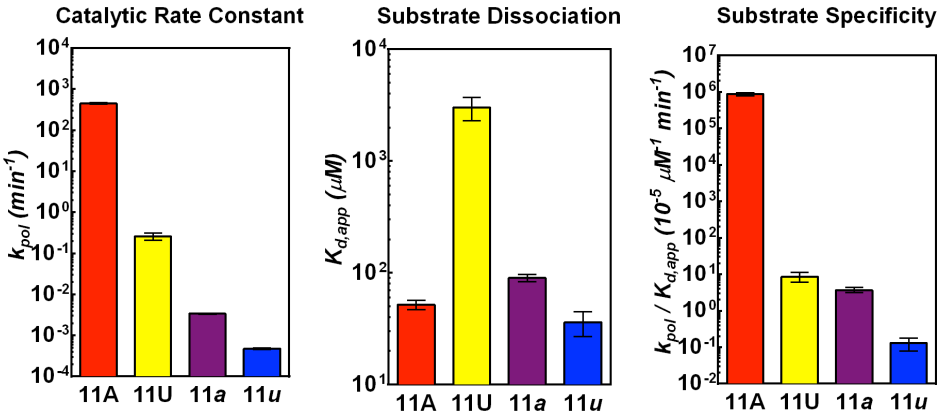

Figure S3. Subsequent nucleotide extension specificity for canonical and unlocked nucleotides (the second checkpoint step). Rate constants (k_{pol}), substrate dissociation constants ($K_{d,app}$) and substrate specificity constants ($k_{pol}/K_{d,app}$) for 11A, 11U, 11***a*** and 11***u*** extension are shown in red, yellow, violet and blue, respectively.

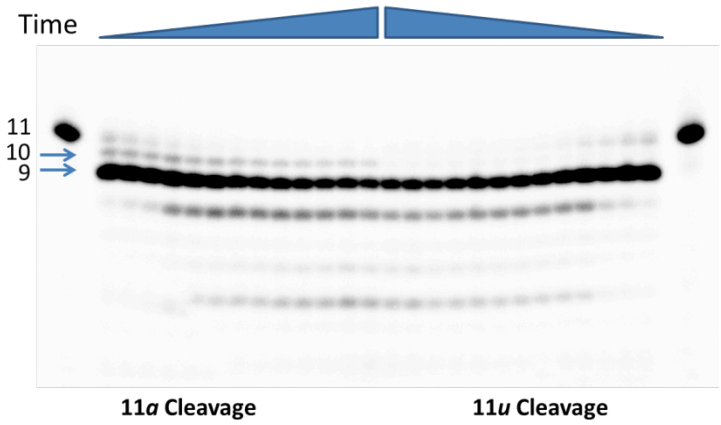

Figure S4. TFIIS-mediated cleavage of scaffolds 11***a*** and 11*u*. Time points vary from 30s to 2hr. The presence of an n-1 product (10nt) at early time points for both 11A:dT and 11***a***:dT scaffolds indicates the existence of a Pol II pre-translocation state. In contrast, cleavage from scaffolds 11U:dT and 11***u***:dT only resulted in an n-2 product (9nt) but not an n-1 product (10nt) at early time points, suggesting the existence of the backtracked Pol II but a lack of the pre-translocation state.

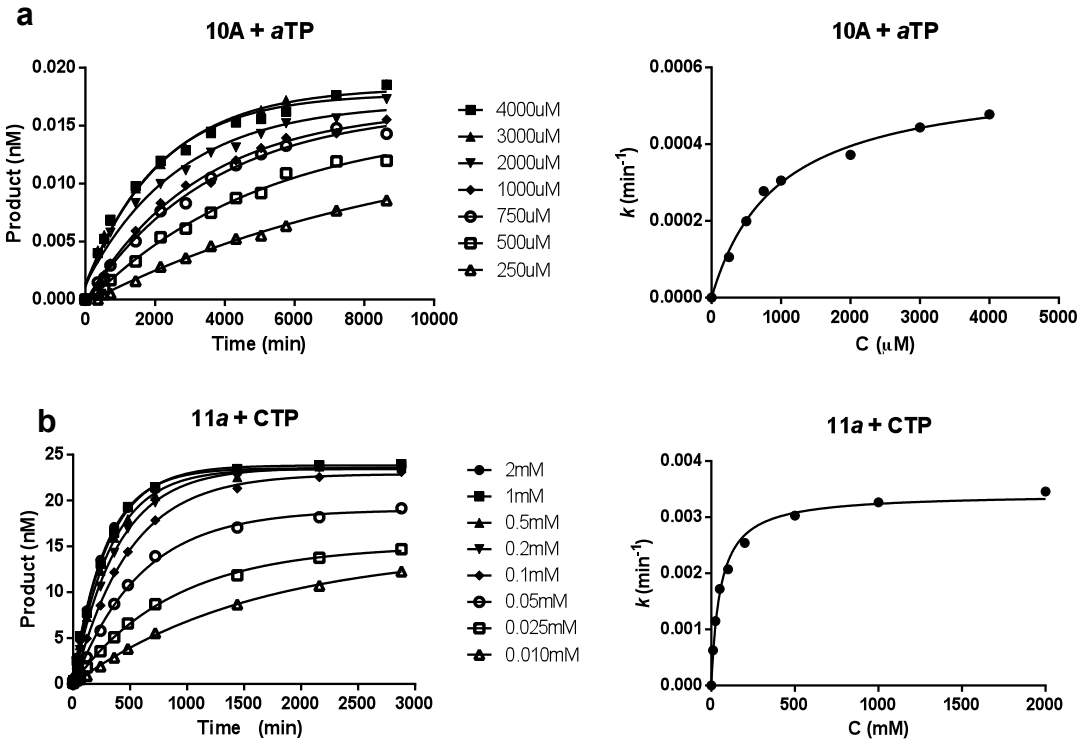

Figure S5. Representative kinetic fitting curves of UNA-involved single nucleotide incorporation. a) Kinetic curves of unlocked nucleotide incorporation (10A + ***a***TP). b) Kinetic curves of subsequent nucleotide extension after the unlocked nucleotide (11***a*** + CTP).

Supplementary References:

[1] a) D. Wang, D. A. Bushnell, K. D. Westover, C. D. Kaplan, R. D. Kornberg, *Cell* **2006**, *127*, 941-954; b) N. Langkjaer, A. Pasternak, J. Wengel, *Bioorg. Med. Chem.* **2009**, *17*, 5420-5425.

[2] a) M. W. Kellinger, C. X. Song, J. Chong, X. Y. Lu, C. He, D. Wang, *Nat. Struct. Mol. Biol.* **2012**, *19*, 831-833; b) M. W. Kellinger, S. Ulrich, J. Chong, E. T. Kool, D. Wang, *J. Am. Chem. Soc.* **2012**, *134*, 8231-8240; c) D. Wang, G. Zhu, X. Huang, S. J. Lippard, *Proc. Natl. Acad. Sci. U S A* **2010**, *107*, 9584-9589.